

Figure S1. PhCM1 and PhCM2 CDS alignment using the Align X program in Vector NTI advance 10.3.0 software package (Invitrogen; Carlsbad, CA, USA). PhCM1 and PhCM2 share 46.1% identity at the nucleotide level.

Figure S2. qRT-PCR transcript accumulation analysis of PhCM1 and PhCM2 in petunia. Spatial analysis used root, stem, stigma, anther, leaf, petal tube, petal limb, and sepal tissues of MD harvested at 16:00 h (a). The spatial experiment consisted of one biological replicate used for sqRT-PCR and one separate biological replicate with two technical replicates per biological replicate. Floral developmental analysis used MD flowers from 11 sequential stages at 16:00 h (b). The developmental analysis consisted of two biological replicates separate from the biological replicates used for the sqRT-PCR with three technical replicates. Ethylene treatment (2 μ l L⁻¹) analysis used excised MD and 44568 whole flowers treated for 0, 1, 2, 4, and 8 h (c). The ethylene treated series consisted of one biological replicate used in the sqRT-PCR with two technical replicates per biological replicate. PhFBP1 and Ph18S were used as references throughout these experiments.

Figure S3. Schematic representation and nucleotide comparison of RNAi region used for the production of petunia PhCM1 RNAi transgenic lines. 213 bases at the 3' end of the coding sequence of PhCM1 were chosen for the RNAi construct. This region shared 58.2% identity with the corresponding nucleotide region from PhCM2.

Figure S4. sqRT-PCR transcript accumulation analysis in floral tissues of three independent T1 ir-PhCM1 lines. MD, 2-4, 24-9, 33-9 were used with primers specific for floral volatile benzenoid/phenylpropanoid, shikimate, and phenylpropanoid transcripts. The number of cycles used for amplification of each transcript is shown on the right. Ph18S was used as a loading control in all cases.

Figure S5. sqRT-PCR transcript accumulation analysis in floral tissues of two independent, homozygous T2 ir-PhCM1 lines. Individuals and biological replicates from MD, 24-9, 33-8 were used with primers specific for PhCM1. The number of cycles used for amplification of each transcript is shown on the right. Ph18S was used as a loading control.

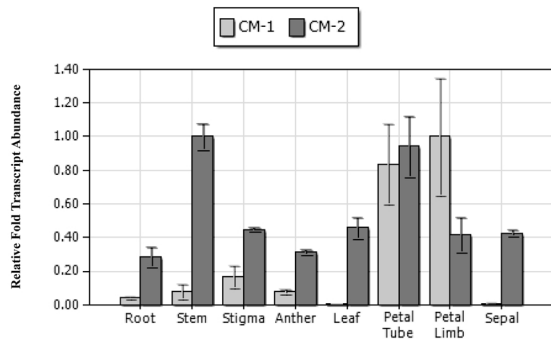
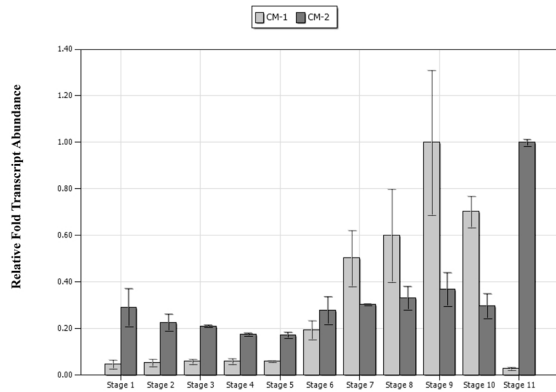
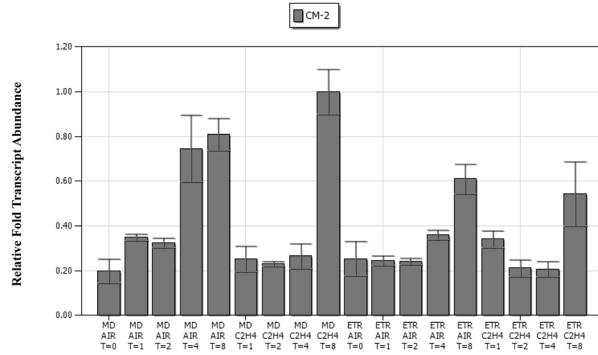
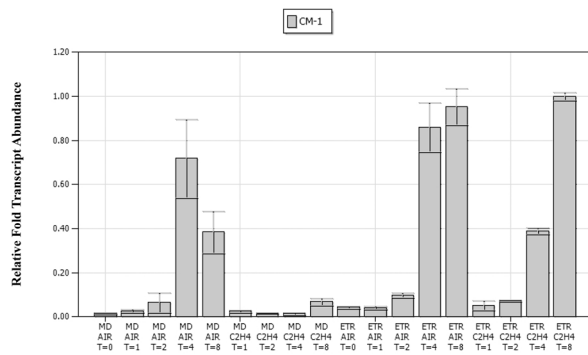
Figure S6. Physiological comparison between MD and representative independent T2 ir-PhCM1 homozygous lines 24-9 and 33-8 in 9 week old petunia seedlings (mean \pm SE; n = 5).

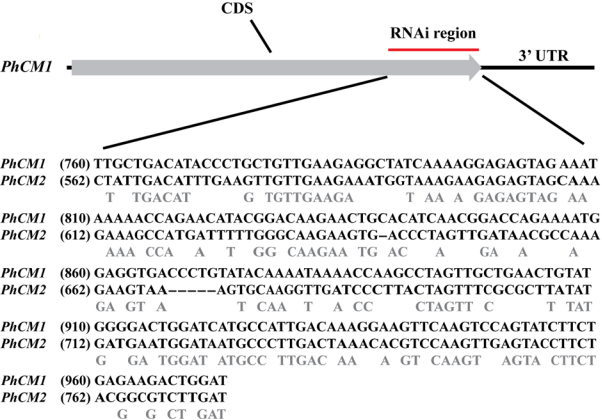
Figure S7. Stem cross-sections (between 7–8 node from apical meristem) from 9 week old petunias stained with Phlorogucinol. Shown are MD and representative individuals from two independent ir-PhCM1 homozygous T2 lines, 24-9 and 33-8. Pictures are from light microscopy at 4 \times on a Leica MZ 16F and are representative of three biological replicates.













































Table S1. Functional complementation of CM-deficient *E. coli* KA12/pKIMP-UAUC. M9c minimal media was used for all experiments and supplemented with 20 μ g ml⁻¹ of L-phenylalanine and L-tyrosine where stated. Antibiotics used were chloramphenicol [Ch] (30 μ g ml⁻¹) for selection of the pKIMP plasmid and carbenicillin [Ca] (100 μ g ml⁻¹) for selection of the pET-32 plasmid. KA12/pKIMP-UAUC is not a λ DE3 lysogenic *E. coli*, so bacteriophage CE6 (Novagen, cat# 69390) infection was used to induce transcription from the pET-32 T7 promoter where stated and no CE6 administered (NA) where stated. Transformants were incubated at 37°C for two days, and growth was scored as a plus (+) or minus (–). A sample of positive colonies was picked and colony PCR was performed for confirmation of pET-32-CM1 or pET-32-CM2 plasmids.

Table S2. Gene specific primers used for the transcript accumulation analyses throughout this study.

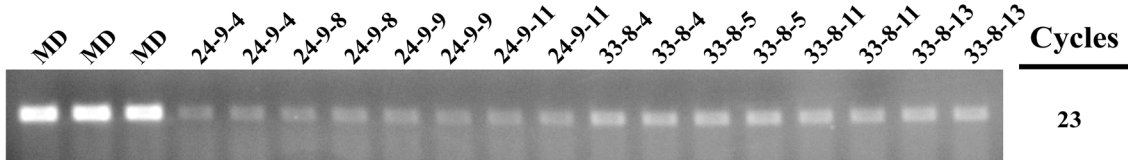
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PhCM1 CDS	(1)	ATGGAACCCCAATTGTTAAGATTCCCTTCTCATACTATAACAAGTTCCATTACTACTAATTCTTCA																																			
PhCM2 CDS	(1)	-----																																			
Consensus	(1)	-----																																			
	(67)	67	80	90	100	110	120	132	Section 2																												
PhCM1 CDS	(67)	AGAAATACTACCCTTTTTTACCCCATAAAAAATGGTCACATTTTGTGAAGTTTCAGTTAGTCAAT																																			
PhCM2 CDS	(1)	-----																																			
Consensus	(67)	-----																																			
	(133)	133	140	150	160	170	180	198	Section 3																												
PhCM1 CDS	(133)	TCAAGTAGTAGTATTAAGCATGGCATTAGACCCCTTCAAGCTTCTGCAACTTCTCTTGGACTTGGG																																			
PhCM2 CDS	(1)	-----																																			
Consensus	(133)	-----																																			
	(199)	199	210	220	230	240	250	264	Section 4																												
PhCM1 CDS	(199)	AAT	AAAA	CACAT	AGATG	AGACC	GAA	GTTAC	AC	FCTTGAT	GGT	ATAAGG	C	CTC	TTTAAT	CC	GA																				
PhCM2 CDS	(1)	---	ATGGC	CTGT	GTGAT	TATGAT	GATA	AACTG	AG	FCTTGAT	TTTA	ATAAGG	G	TAG	TTTAAT	TGA																					
Consensus	(199)	A	C	G	G	GAT	A	GA	A	A	TCTTGAT	ATAAGG	A	T	TTAAT	GA																					
	(265)	265	270	280	290	300	310	320	330	Section 5																											
PhCM1 CDS	(265)	CAAGAAGATAGCATATAATATTCAGCCCTTG										G	SAGAGA	GC	TCT	AGT	AC	TG	TAC	AAT	G	CGG	GAC	AT	T												
PhCM2 CDS	(64)	CAAGAAGATACCATATTTTCAACCTTA										T	SAGAGA	AT	CA	AG	GA	AA	T	CCA	ATA	AAT	T	CAC	CTT	G	T	A									
Consensus	(265)	CAAGAAGATA										CAT	AT	TTCA	CC	T	T	GAGAGA	A	T	C		AAT	C													
	(331)	331	340	350	360	370	380	396	Section 6																												
PhCM1 CDS	(331)	GTCTCTG										ATG	TTT	TTC	AT	GG	AT	TG	GG	TT	CAT	GG	CT	CT	TG	GG	T	AG	T	AT	T	G	AG	AA	AC		
PhCM2 CDS	(130)	AAAAACC										AT	CT	TG	AT	TT	TCC	AG	AT	TT	AC	AG	CT	CT	TG	GG	T	AG	T	AT	T	G	AG	AA	AC		
Consensus	(331)	A		AT	TT	TTG	G	T	TG	TTT		GG	TC	TTG	T	AGTA	T	TC	AGAAAC																		
	(397)	397	410	420	430	440	450	462	Section 7																												
PhCM1 CDS	(396)	TGAA										AAG	CTT	CC	AG	C	CG	TT	GG	AA	GA	T	AA	AGC	CC	T	GAT	GA	GC	AC	CC	AT	TCT	TT	CC	AA	AGT
PhCM2 CDS	(195)	GAAAGCT										CTT	CC	AT	CC	AG	TT	GG	T	AG	GA	T	AA	AGC	CC	T	GAT	GA	GC	AC	CC	AT	TCT	TT	CC	AA	AGT
Consensus	(397)	GAA		CTTCA		C	A	GGTTGG	AG	TA		A		CC	GA	GA	A	CC	TTCT	CCA	A																
	(463)	463	470	480	490	500	510	528	Section 8																												
PhCM1 CDS	(462)	ATT										CCG	AGC	CA	GT	GT	G	CCACC	CAT	GC	AT	G	TAC	CCA	AAG	GTT	CT	GC	AC	CC	AA	TT	GG	T	GAT	T	CAAT
PhCM2 CDS	(261)	CTT										GCCT	CCCT	CA	AT	CG	AT	CCACC	CT	AGTA	AT	GT	CCA	CCA	GTT	TT	GC	AT	CCA	GT	AG	AG	AG	AT	GC	AT	CA
Consensus	(463)	TT	CC	G		CA	T	T	CCACC	A		A	T	CCA		GTT	TGCA	CCA	T	GC	GA	TC	AT														
	(529)	529	540	550	560	570	580	594	Section 9																												
PhCM1 CDS	(528)	TAATAT										CAATG	TC	AAA	ATAT	GGGA	AA	TG	AT	CT	CGGGA	AT	CT	CT	CCA	AGAT	TAGTA	AA	GA	GA	AG	AG	AG	AG	AG	AG	
PhCM2 CDS	(327)	CAATAT										AAATG	AG	AA	ATAT	TGG	AT	GT	TT	AT	CT	CAAATC	AG	TA	CT	CT	CCA	ACT	ATT	CT	GT	ACT	GAG	GC	GC	GC	
Consensus	(529)	AATAT	AATG	AA	ATAT	GGA	T	TA	T		A	T	CT	CCA	ATT	A	GA	G																			
	(595)	595	600	610	620	630	640	660	Section 10																												
PhCM1 CDS	(594)	TGATGA										TGGTGGT	TAC	CGAT	CTAG	AGCA	TTT	TGTGA	CAC	CT	ATAT	GCG	TGC	CAGGC	CC	TG	CT	G	AG	AG	AG	AG	AG	AG	AG	AG	
PhCM2 CDS	(393)	TGATGA										AGGAAC	TATG	CACTAG	TGCTC	CTGTGAT	AT	CAGTT	TGCT	TGC	CAGGC	AA	CT	CT	GA	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	
Consensus	(595)	TGATGA	GG		TA	G	A	CTAC	GC	G		TGTGA	A	T		T	TGCAGGC	T	TC	A	AG																
	(661)	661	670	680	690	700	710	726	Section 11																												
PhCM1 CDS	(660)	AAT										CACTAT	TGG	AAATTTGTT	TGCTGA	AT	CAAAAT	AT	CA	ACC	T	CAC	CAG	AG	AT	CT	TATA	AT	G	TGC	TGC	TGC	TGC	TGC	TGC		
PhCM2 CDS	(459)	GAT										CACTAT	TGG	AAATTTGTT	TGCTGA	AT	TAAT	AT	CA	ACC	AT	TGCT	T	GA	C	AA	TATA	CAC	ACT	ACT	ACT	ACT	ACT	ACT	ACT		
Consensus	(661)	ATTCACTAT	TGG	AAATTTGTT	TGCTGA	G	AAAT		G	G	T		C	GA	G	TATA	C																				
	(727)	727	740	750	760	770	780	792	Section 12																												
PhCM1 CDS	(726)	TATT										AGAG	CC	CA	AGATAG	AAATG	GC	TT	TAATG	GATT	G	CT	TGACAT	ACCCT	CT	T	TTT	GAAGA	AGG	CTAT	T						
PhCM2 CDS	(525)	TATT										CTT	GCT	CAG	ATAG	GGG	TG	CTC	TAATG	AAAC	AT	TGACAT	TTGAA	GT	T	TTT	GAAGA	AAAT	GGT	T							
Consensus	(727)	TATT		GC	CA	GATAG		TG		TAATG	A	T	TGACAT		G	TGTTGAAGA	T																				
	(793)	793	800	810	820	830	840	858	Section 13																												
PhCM1 CDS	(792)	CAA										ANG	GAGAGTAG	AAA	TAAAA	CCAGAA	CAT	TAC	GG	CAAGAA	CTG	CACAT	CT	C	ACG	GA	CC	AGAAA	AGAAA	AGAAA	AGAAA	AGAAA	AGAAA	AGAAA			
PhCM2 CDS	(591)	AAA										GAA	GAGAGTAG	CAA	AGAAA	CCATG	ATTTT	GG	CAAGAA	GT	ACCC	T	AGTT	GAT	A	CGCCA	AGCCA	AGCCA	AGCCA	AGCCA	AGCCA	AGCCA	AGCCA	AGCCA			
Consensus	(793)	AA	A	GAGAGTAG	AA	AAA	CCA	A	T	GG	CAAGAA	TG	C	T	A	GA	A	A																			
	(859)	859	870	880	890	900	910	924	Section 14																												
PhCM1 CDS	(858)	TG										AGG	GT	AC	CC	TGTA	TA	CAAAA	TA	AAA	CC	AAGC	CTAG	TT	G	CT	GAA	AC	CT	GAT	AT						
PhCM2 CDS	(656)	AAG										AG	TA	NA	-----	G	T	CAAGG	TG	AT	CC	CTTA	CTAG	TT	G	CT	GAA	AC	CT	GAT	AT						
Consensus	(859)		GA	GT	A			T	CAA	T	A	CC					CTAGTT	C		T	TATG	GA	TGGAT	AT													
	(925)	925	930	940	950	960	970	980	990	Section 15																											
PhCM1 CDS	(924)	GCC										ATTGAC	AA	GG	AG	TT	CAAGT	CC	AGTA	CTT	CT	G	AA	GA	ACT	GT	AT	-----	-----	-----	-----	-----	-----	-----			
PhCM2 CDS	(717)	GCC										CTTGAC	TA	AA	CG	TC	CAAGT	TG	AGTA	CTT	CT	AC	GC	CT	GT	GAT	CAGA	ACAA	CTAA	CTTC	CTTC	CTTC	CTTC	CTTC	CTTC		
Consensus	(925)	GCC		TTGAC	AA		A	GT	CAAGT		AGTA	CTTCT		G	G	CT	GAT																				
	(991)	99	994																																		
PhCM1 CDS	(973)	----																																			
PhCM2 CDS	(783)	TATC																																			
Consensus	(991)																																				

(a)**(b)****(c)**

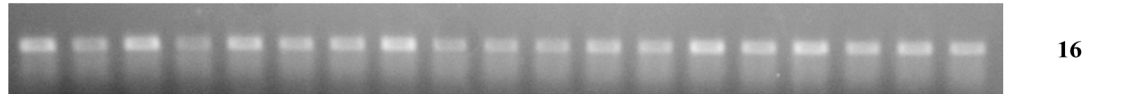


	MD	2-4	24-9	33-9	Cycles
<i>PhODO1</i>					22
<i>PhBSMT1</i>					22
<i>PhPAAS</i>					24
<i>PhIGS1</i>					20
<i>PhPAL2</i>					21
<i>PhPAL1</i>					20
<i>PhPD1</i>					21
<i>PhEPSPS</i>					22
<i>PhCM1</i>					21
<i>PhCM2</i>					26
<i>Ph18S</i>					16

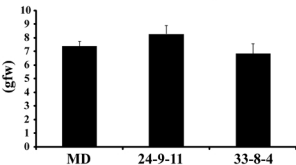
PhCM1



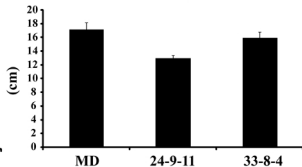
Ph18S



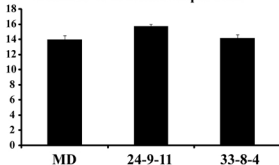
Plant Fresh Weight



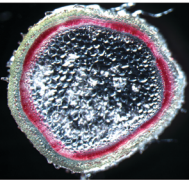
Plant Height



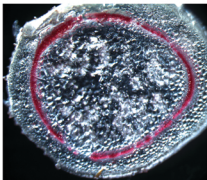
Number of True Leaves per stem



MD



24-9-11



33-8-4



Bacteria/Plasmid	Media	Antibiotic	Treatment	Growth
KA12/pKIMP-UAUC	M9c/Phenylalanine, Tyrosine	Ch	NA	+
KA12/pKIMP-UAUC	M9c	Ch	NA	-
KA12/pKIMP-UAUC	M9c/Phenylalanine, Tyrosine	Ch	CE6	+
KA12/pKIMP-UAUC	M9c	Ch	CE6	-
KA12/pKIMP-UAUC	M9c/Phenylalanine, Tyrosine	Ch/Ca	NA	-
KA12/pKIMP-UAUC	M9c	Ch/Ca	NA	-
KA12/pKIMP-UAUC	M9c/Phenylalanine, Tyrosine	Ch/Ca	CE6	-
KA12/pKIMP-UAUC	M9c	Ch/Ca	CE6	-
KA12/pKIMP-UAUC/pET-32-CM1	M9c/Phenylalanine, Tyrosine	Ch/Ca	NA	+
KA12/pKIMP-UAUC/pET-32-CM1	M9c	Ch/Ca	NA	-
KA12/pKIMP-UAUC/pET-32-CM1	M9c/Phenylalanine, Tyrosine	Ch/Ca	CE6	+
KA12/pKIMP-UAUC/pET-32-CM1	M9c	Ch/Ca	CE6	+
KA12/pKIMP-UAUC/pET-32-CM2	M9c/Phenylalanine, Tyrosine	Ch/Ca	NA	+
KA12/pKIMP-UAUC/pET-32-CM2	M9c	Ch/Ca	NA	-
KA12/pKIMP-UAUC/pET-32-CM2	M9c/Phenylalanine, Tyrosine	Ch/Ca	CE6	+
KA12/pKIMP-UAUC/pET-32-CM2	M9c	Ch/Ca	CE6	+

Reference Number	Target	Direction	Primer Sequence (5'→3')
sqRT-PCR			
AJ236020	<i>Ph18S</i>	Forward	TTAGCAGGCTGAGGTCTCGT
		Reverse	AGCGGATGTTGCTTTTAGGA
AY233465	<i>PhBSMT1</i>	Forward	AGAAGGAAGGATCATTACCA
		Reverse	TATTCGGGTTTTTCGACCAC
EU751616	<i>PhCM1</i>	Forward	GCCATTGACAAAGGAAGTTCA
		Reverse	TGTTCCAAACTTGAAAATTACATCA
EU751617	<i>PhCM2</i>	Forward	CCATCTTCTTGTTTCCTGA
		Reverse	TCACAGGCAGCAGTAGTTGC
M21084	<i>PhEPSPS</i>	Forward	CGATGATCACAGGATGGCCATGG
		Reverse	CCCATTGTGGTTCTGATACCAG
DQ372813	<i>PhIGS1</i>	Forward	GCCTATGTCATGCCATTGAA
		Reverse	TGCTTTAATTGTGTAGGCTGC
AY705977	<i>PhODO1</i>	Forward	TTCAATTGGCTTTCGGGTTA
		Reverse	AGGCACCTTGGACTCTTCG
DQ243784	<i>PhPAAS</i>	Forward	TCCTTGTAGTTCTAGTACTGCTGGAA
		Reverse	TCAACAGCAGTTGTTGAAGTAGTTC
AY705976	<i>PhPAL1</i>	Forward	GCTATGAATGAAGGAAAGTTGG
		Reverse	CACAATCTTTCATACAAACCC
CO805160	<i>PhPAL2</i>	Forward	CCGAGCTGTTGACAGGAGAAGG
		Reverse	TAACCAGACTACTAAAGTTCAGC
SGN-U207570	<i>PhPDI</i>	Forward	ACATCGCAGCCAATAACCTT
		Reverse	CCCACATTTGCATCATCAAC
qRT-PCR			
AJ236020	<i>Ph18S</i>	Forward	TGCAACAAACCCCGACTTCT
		Reverse	AGCCCGCGTCAACCTTTTAT
EU751616	<i>PhCM1</i>	Forward	CCCTGATGAGCACCCATTC
		Reverse	ACTGCATGGGTGGCAACAC
EU751617	<i>PhCM2</i>	Forward	AACTTGCCTGCCTCAATCGT
		Reverse	GGATGCAAACTGGTGGACAT
M91190	PhFBP1	Forward	TGCGCCAAC TTGAGATAGCA
		Reverse	TGCTGAAACACTTCGCCAATT